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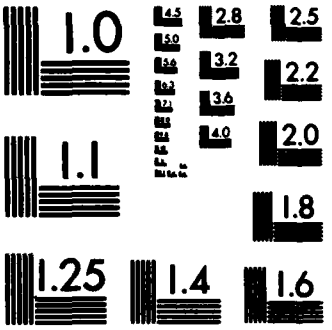

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ANNUAL REPORT

Craig R. Hassler and Larry G. McCoy

May, 1982

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DADA17-69-C-9118

BATTELLE
Columbus Laboratories
505 King Avenue
Columbus, Ohio 43201

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Implant Materials	Bioresorbable Ceramics	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Research studies were conducted to produce and evaluate a high-quality directional porosity resorbable calcium phosphate ceramic material for use in the management of hard tissue avulsive wounds and orofacial fractures. The previous year's efforts demonstrated that directional porosity would allow adequate ingrowth of bone through the biomaterial prior to loss of mechanical integrity of the biomaterial.

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The overall objective is to produce a completely resorbable biomaterial which will promote bone formation and after the bone remodeling, biodegradation process be completely replaced by bone. It should be pointed out that the dynamics of this situation are complex. The biomaterial should allow bone ingrowth and provide mechanical integrity during the remodeling, biodegradation process. The simultaneous dissolution of the biomaterial and bone formation need to proceed in a parallel and controlled fashion, so that mechanical integrity of the area under repair is not lost. In early studies, omnidirectional structural material would, depending upon chemical composition, either: not entirely biodegrade, or would degrade until mechanical integrity was lost. Preliminary unidirectional materials, as reported in our previous annual report, were free of these problems; ingrowth and subsequent bioresorption without loss of implant integrity was noted. The material utilized last year proved the concept, but pore density was low and mechanical strength was less than desired.

The advanced materials produced for this present investigation were calendered or rolled to form a serrated surface and then stacked and sintered together to form a unique undirectional porosity as required by the particular implant situation. The technique allows directional porosity material to be formed in blocks of high-strength material with continuous pores of large diameter. The pores can be specifically oriented, and the surrounding material can be made dense enough to provide a high-strength scaffold.

Since this is a major new direction for processing tricalcium phosphate, several preliminary studies were performed. These studies included: preparation and characterization of tricalcium phosphate, studies of ceramic-organic mix formulation, development of sheet forming techniques, and production of embossed sheets.

Implants approximately 8 mm in diameter were placed bilaterally in the calvaria of the animals and were evaluated for periods of 3, 6, 9, and 12 months. At the time of this report, the 12-month animals were still on test. At each time period, a portion of the animal population was necropsied and analyzed by histologic and radiographic methods. The results of the *in vivo* study indicate that unidirectional porosity will allow bone ingrowth and biodegradation without loss of implant integrity. These results are in agreement with our previous unidirectional material experiments. The analysis suggests that the concept of large diameter porosities, oriented in the direction of desired bone growth, is feasible. The material used in this study demonstrates a practical method of producing the desired material with adequate pore density. However, the materials' mechanical properties need improvement, especially at the interfaces where the embossed sheets are sintered together.

MANAGEMENT OF HARD TISSUE AVULSIVE WOUNDS AND MANAGEMENT OF OROFACIAL FRACTURES

by

Craig R. Hassler and Larry G. McCoy

SUMMARY

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FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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BACKGROUND, PROBLEM AND APPROACH

Historically, various techniques have been employed for the repair or treatment of osseous diseases, defects, and wounds. Autogeneous bone grafting remains the most satisfactory approach, but is not without the disadvantages associated with double surgeries, limits in structural properties, and the limitations imposed on the repair of massive osseous defects.

Since April, 1970, Battelle's Columbus Laboratories has been conducting research under contract with the Dental Research Division, U.S. Army Medical Research and Development Command, on the development of resorbable ceramics for potential application in the repair of hard tissue avulsive wounds. The basic materials have been calcium phosphates. These materials were selected because they contain two of the essential elements of the natural bone mineral phase, calcium hydroxyapatite.

In vivo studies were conducted initially at the U.S. Army Institute of Dental Research (USAIDR), using the sintered porous materials and slurries prepared at Battelle from tricalcium phosphate $Ca_3(PO_4)_2$ and other calcium orthophosphate powders $CaHPO_4$ and $Ca(H_2PO_4)_2$, to evaluate the potential use of calcium phosphates to both facilitate repair of bone defects and to determine the best material for future exploration[1-3]. The implant studies indicated that calcium phosphates consisting essentially of the mineral phases $Ca(PO_3)_2$, $Ca_3(PO_4)_2$, and $CaHPO_4$ are well tolerated by the tissue, appear to be nontoxic, are resorbable, and permit rapid invasion of new bone.

Of the various porous calcium phosphate materials investigated, tricalcium phosphate, $Ca_3(PO_4)_2$, was selected for continued development and evaluation since it was easy to fabricate and was found to be both biocompatible and resorbable. Emphasis has been directed toward producing porous materials consisting of single-phase tricalcium phosphate[4-7]. Research on granular formations of tricalcium phosphates (TCP) continued at USAIDR. Basic research at Battelle-Columbus was focused on producing practical large segment replacement implants from TCP.

To provide basic resorption rate data on the in vivo behavior of solid tricalcium phosphate bioresorbable ceramics, implant studies were initiated in 1975 at Battelle-Columbus using the rabbit calvarium model[8]. Early samples of tricalcium phosphate were implanted as a control and samples of two new materials were implanted for comparative observation. These new materials were prepared using the improved processing techniques derived in previous materials development studies and represented significant improvements in the structural characteristics of porous tricalcium phosphate. The characterization of the materials involved and the results of the in vivo studies were the subject of the Fifth Report[8].

These results indicated that the improved material exhibited significant increases in resorption rate. In fact, the material resorbed so rapidly that after the ninth month the implant appeared to be granulated and was invaded with connective tissue. This result does not imply lack of biocompatibility, but does suggest that such rapid degradation can be deleterious in stress-bearing situations. It was not known then whether the enhanced resorptivity resulted from achieving a Ca/P ratio closer to the theoretical for tricalcium phosphate, or from the improvements in the structural characteristics of the material.

To determine the effects of structural variations on resorption rate, experimental porous implants were prepared using a single tricalcium phosphate powder with different pore size distribution. Three materials were prepared for in vivo evaluation. These studies demonstrated that orientation of pore structure is a more important variable than pore size distribution.[9] The study indicated that a higher density material of the stoichiometric chemistry with directional porosity is probably the desired material.

The seventh report[10] demonstrated that the concept of directional porosity could provide the predicted result, namely, adequate ingrowth of bone to provide mechanical integrity prior to loss of mechanical integrity of the tricalcium phosphate. These results were corroborated by Tortorelli.[11] The material used in these experiments was far from ideal; consequently, a better method of production was sought. The ideal material should minimally inhibit the ingrowth of bone; consequently, large pores and a

high pore density were desirable. The ideal material should also be of high strength and should have mechanical properties approaching bone. Consequently, a material of high density in the non-porous regions was sought. It was also deemed desirable to have a material that could be readily manufactured with the pore alignment and size required for a particular application. The fulfillment of these requirements were the goals of the development and evaluation outlined in this report.

MATERIALS AND METHODS

A major effort this year was the development of an improved unidirectional porosity material. This material was calendered (or rolled) to form a serrated surface and then stacked and sintered together to form a unique unidirectional porosity as required by the particular implant situation. Since this is a major new direction to the processing of tricalcium phosphate, several preliminary studies were required prior to the actual formulation of the final material. The tasks completed for the forming of the unidirectional porosity material are outlined in the following paragraphs.

Task 1. Prepare Tricalcium Phosphate Powder

Certified ACS calcium carbonate was combined with certified ACS phosphoric acid to form a powder precipitate by slowly mixing these two components into distilled water at 180 F. Once dried in air, the powders were dried under vacuum, 220 F, overnight. Approximately 4800 gms of material in six batches were prepared in this manner.

Task 2. Characterize the Tricalcium Phosphate Powder for Chemical Composition and Physical Properties

X-ray diffraction analysis of the above prepared powder revealed it to be hydroxyl-apatite with a trace of monetite. The powder surface area was 12 m^2/gm, which would mean a particle size in the submicron range. To crush the large, hard agglomerates the hydroxyl-apatite was dry ball milled for 2

hours in a polyethylene container with aluminum oxide balls. To determine the optimum calcining temperature to convert hydroxl-apatite to whitlockite, five 100 gm samples were calcined for 3-1/2 hours at 1400, 1500, 1550, 1600, and 1700 F, followed by an X-ray diffraction analysis and a surface area test. The maximum surface area of 8.2 m^2/gm and simultaneous maximum conversion to whitlockite occurred at 1550 F. Table 1 shows X-ray diffraction results at the various temperatures evaluated.

To break up agglomerates formed during calcining, the powder was ball milled with hexane for 12 hours in a polyethylene jar with aluminum oxide balls. Table 2 lists characterizations done on two powders.

TABLE 1. X-RAY DIFFRACTION - RELATIVE PATTERN STRENGTH

Calcine Temperatures, °F	Hydroxyl-apatite	Monetite	Whitlockite	α $Ca_3(PO_4)_2$	Surface Area (m^2/gm)
	100	25			12.07
1400	85		100	50	11.28
1500	18		100		10.38
1550	2		100		8.2
1600	2		100		2.98

TABLE 2. DENSITY RESULTS

	Calcined 1550 F	Calcined 1550 F, Then Ball Milled
Bulk density (percent theoretical)	10.6%	16%
Tap density (percent theoretical)	14.1%	23.5%
Surface area m^2/gm	8.2	11.6

To further characterize this powder, pellets were pressed and sintered at 2000, 2050, and 2100 F. In this temperature range, tricalcium phosphate has a destructive transformation from β to α form, with the alpha form being of lower density. The highest sintered density was obtained by heating to 2050 F in one hour, holding for 2 hours and cooling slowly (overnight) to room temperature. This yielded a sintered pellet with 92 percent theoretical density and a linear shrinkage of 12 percent.

Task 3. Conduct Preliminary Study on Ceramic-Organic Mix Formulation to Develop Sheet Forming Techniques

An alumina powder, A-16SG, made by Alcoa, was chosen in this preliminary study since its particle size distribution and surface area, 10-12 m^2/gm is similar to the tricalcium phosphate material being used. Various compositions containing A-16SG alumina, an organic solvent, binder, plasticizer, and a deflocculant were mixed, milled for 24 hours, and cast. Once a suitable composition - one which dried easily without cracks and had excellent flexibility - was found, the tricalcium phosphate material was used in place of the A-16SG alumina. The composition was a polymeric formulation containing appropriate dispersing agents.

Task 4. Produce Embossed Sheets

An embossing tool was machined from a teflon rod with circular grooves of the dimensions required. During the drying of the tricalcium phosphate tape cast piece, the embossing tool was rolled over the surface, producing the grooves. Figure 1 shows the embossing tool as applied to the tape cast material to form fluted sheets.

Task 5. Assembly of Implant Structure

After the fluted sheets dried in ambient conditions for 15 to 30 minutes, 3/4-inch squares were cut from the sheets by using a razor knife. An Exacto saw was used to cut small interconnecting channels through the embossed

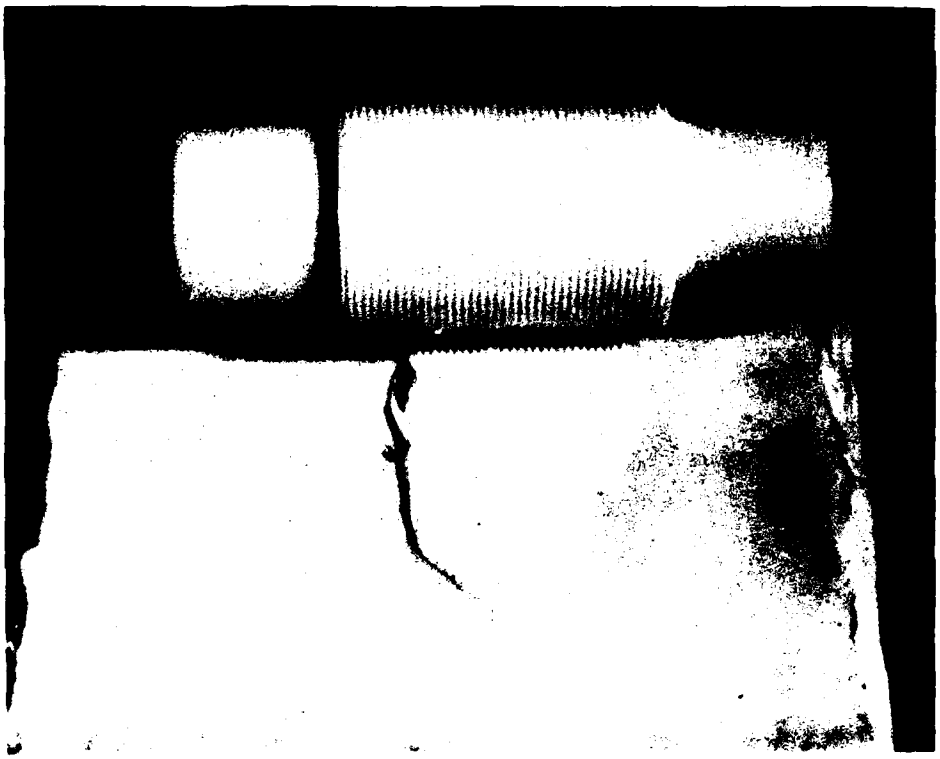

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FIGURE 1. EMBOSSING TOOL BEING USED TO PRODUCE FLUTED SHEETS FROM TAPE CAST MATERIAL

The simple rolling action of the embossing tool over flexible tape cast material produces fluted sheets, which form the basis of pores. The next fluted layer stacked on top will complete the directional pores. The various layers, stacked alternately at right angles, are fused together by sintering. The sintering also removes the organic binders and shrinks the matrix to the desired dimensions.

ribs perpendicular to the main channels. The ceramic-organic slurry was painted on the underside of the tricalcium phosphate square, and the squares were stacked in alternate directions at right angles. Finally, a 20-gauge drill was used to produce a connecting hole between layers of the matrix.

Task 6. Cure and Remove the Organic Binders from the Matrix

Removal of the organic binders and the sintering of the matrix structure was accomplished in one step (see Task 7).

Task 7. Sinter the Matrix Structure

The embossed tape cast pieces were heated in 1 hour to 2050 F and held there for 2 hours, producing pieces with a density of ~50 percent of theoretical. The organic binders were removed from the pieces without producing cracks. However, X-ray diffraction analysis revealed the presence of a small proportion of alpha tricalcium phosphate in the sintered specimens. Because of the destructive nature of the α to β transformation, the sintering temperature was decreased to 2000 F, resulting in a 100 percent β tricalcium phosphate. This should result in higher strength with little or no reduction in density. Figure 2 shows the finished material cut into rectangular segments. These segments were subsequently cut into circular segments for *in vivo* analysis.

Task 8. Characterization of the Matrix Structure

To characterize the matrix structure, X-ray diffraction, Optical Emission Spectroscopy, and fired density measurements were conducted. X-ray diffraction analysis proved the matrix structure to be 100 percent β tricalcium phosphate. Optical Emission Spectroscopy results are listed in Table 3.

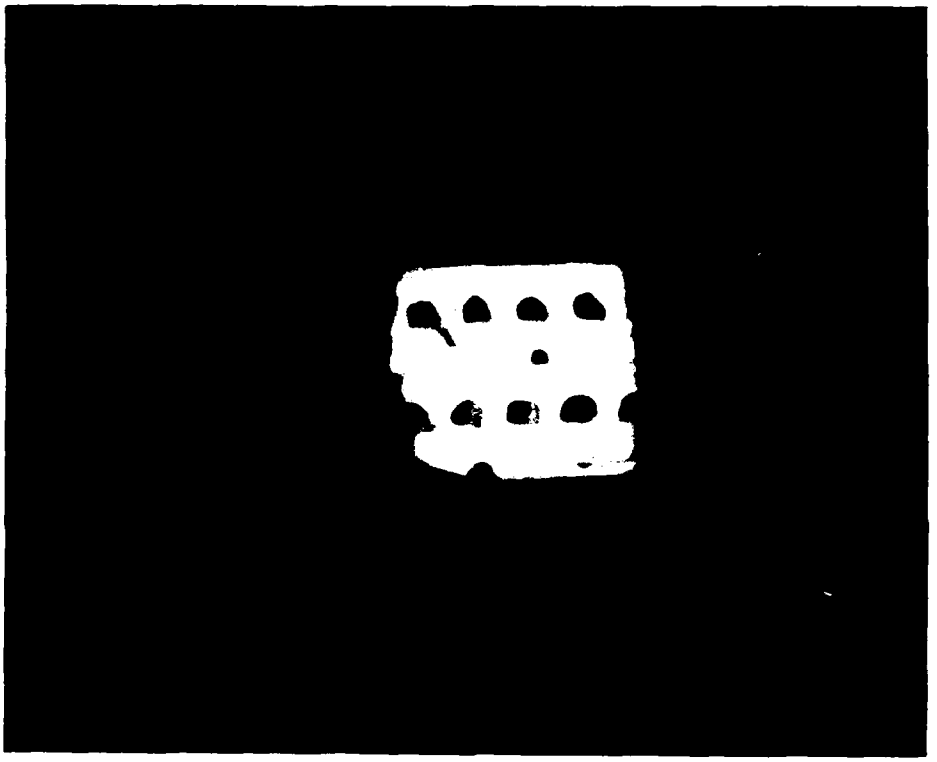

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FIGURE 2. FINISHED MATRIX MATERIAL

The multilayered material is shown in this photograph with two rows of pores shown going into the plane of the photo. These pore layers are alternated with pores oriented at right angles which are not shown in the figure.

TABLE 3. OPTIC EMISSION SPECTROSCOPY RESULTS

Element	Weight Percent as Metal
Ba	T
B	T
Si	.01
Fe	T
Mg	T
Al	.05
Mo	T
Cu	T
W	.02
Na	.02
Li	T
Sr	.03

T-Trace

Mercury displacement showed that the fired density of the matrix was 57.4 percent of theoretical, and the fired density of the laminated implant structure was approximately 35 percent of theoretical.

Although the overall (bulk) density of the material is lower than the previous random porosity implant materials (48-52 percent), the material does appear to have a higher transverse strength. However, there is a tendency to delaminate between layers because of incomplete bonding. Processing improvements are needed to increase strength and achieve better bonding. An increase in bulk density back to the 50 percent range may be necessary to achieve these improvements. This will necessitate developing tape compositions having higher loading of TCP or the adoption of alternative processes.

Task 9. Preparation of Implant Materials for USAIDR and University of Alabama Clinical Programs

Several physically different tricalcium phosphate materials for clinical investigations were produced for Colonel Snyder (USAIDR) and Dr. Jack Lemmons (University of Alabama).

For Colonel Snyder, 250 vials containing sterile granular material (-200/+325 mesh) were produced for use in clinical periodontal studies. To

prepare the powder, a batch of calcined tricalium phosphate, $Ca_3(PO_4)_2$ (Lot C91A), was die pressed to form two-inch diameter by 1/8-inch-thick disks. After firing at 2000 F for 2 hours, the sintered disks were crushed in an alumina mortar and pestle. The resulting powder was sieved to recover the -200/+325 mesh size fraction. To avoid possible contamination during secondary handling the powder was packaged in small (1/6 gram) unit dose vials. After filling, the vials of powder were dry heat sterilized at 500 F for 2 hours. Randomly selected vials have been analyzed for purity to verify the chemistry of the contents and to confirm sterility.

For Dr. Lemmons, a random porosity material was produced in five forms. A minimum of 100 grams of each of the four granular materials and six rods of the requested size were prepared. To prepare the porous material, a batch of calcined tricalcium phosphate powder, $Ca_3(PO_4)_2$ (Lot E69), was blended with -40/+100 mesh napthalene. The material was isopressed as a rod at 50 Kpsi. The napthalene was very slowly evaporated and then the rods sintered to 2000 F for 2 hours. To form the four different particle sizes several of the rods were crushed and screened. The granules were prepared in the following sizes:

Size Requested (millimeter)	Mesh	Size Delivered (millimeter)
3	-6/+8	$2.36 < x < 3.33$
2	-8/+10	$1.65 < x < 2.36$
1	-10/+20	$0.83 < x < 1.65$
-40/+100	-40/+100	$0.104 < x < 0.42$

Randomly selected powder was analyzed for purity and to verify the chemistry of the contents. This material was not requested to be sterile. X-ray diffraction determined the powder to be 100 percent β $Ca_3(PO_4)_2$. No second phases were detected.

EXPERIMENTAL ANIMAL STUDIES

This portion of the report details the various research procedures which are used in our laboratories to evaluate biodegradable materials. The procedures include histology, and radiography

Research Protocol

In order to test the biodegradation of large tricalcium phosphate segments, a special experimental model has been devised in this laboratory. We utilize the calvarium of a mature, male, New Zealand White rabbit with a minimum weight of 8 pounds. The calvarium has been found to be an excellent test implant site for this biomaterial. Since stresses upon the calvarium are not extraordinarily high, external stabilization is not required. Consequently, confusing effects which might be due to stabilization devices are not seen. Of greater importance is the fact that this implant site provides the researcher with a large, relatively uniform area for various simultaneous studies such as periodic radiography, multiple histologic analyses, etc.

Standard aseptic surgical technique was used to expose the calvarium of the anesthetized animal. A circular, 8 mm diameter, portion of the calvarium was osteotomized bilaterally from the animal with no attempt to salvage the periosteum overlying the excised area. The tricalcium phosphate implants were interference fit. The skin incision was closed and the animal was treated with a prophylactic antibiotic. Twelve animals were randomly separated into four experimental groups. The experimental groups consisted of sacrifice dates, 3, 6, 9, and 12 months post implant. Since two implants were placed in each animal, four samples were available for analysis at each sacrifice interval. In addition, two control animals were prepared. These animals had bilateral circular voids 8 mm in diameter.

The animals were radiographed at 3-month intervals until the time of necropsy and the excised skulls were radiographed post-necropsy. The implants were radiographed prior to surgery. Also, a step wedge was incorporated into all radiographs to facilitate comparisons. The histologic technique consisted of embedding portions of the excised calvarium-tricalcium phosphate complex in

methylmethacrylate and sectioning. Half of each excised sample was stained with basic fuchsin prior to sectioning. During the experiment, rabbits were stained at time zero and 3-month intervals with one of the following vital bone growth markers: tetracycline 60 mg/kg, DCAF 20 mg/kg and xylenol orange 90 mg/kg. The other half of the above-mentioned sample was left unstained and sectioned for ultraviolet bone growth analysis utilizing these previously injected vital bone growth markers.

At the time of this report, the animals had been necropsied up through month nine of the protocol. Histology was available for analysis through month six.

Radiographic Examination of Tricalcium Phosphate Biodegradability

Radiographs of the rabbits were taken at time zero, 3-month intervals and of the excised skull after necropsy to monitor the biodegradation of the tricalcium phosphate implant. These high resolution radiographs were obtained using fine-grained industrial X-ray film and a Picker Industrial X-Ray Unit.

Representative of the results are the radiographs of rabbit D-81. Figure 3 shows *in situ* tricalcium phosphate implants 21 days post implant. Note that the implants are readily apparent in the animals' calvarium. In this "live" X-ray detail of the directed porosity of the implant is not distinct. The radiodensity of both implants appears equivalent.

Figure 4 is a radiograph of the same animal (D-81) 3 months post implant. The overall radiodensity of the implants is lower, suggesting some bioresorption within the samples. The right implant is somewhat obscured by the angle of this radiograph.

By six months, there is a dramatic alteration in the appearance of the samples. Figure 5 illustrates the 6-month radiograph of rabbit D-81. The samples are difficult to locate in the radiograph. This suggests that the radiodensity of the samples is approaching that of the surrounding bone and further suggests additional bioresorption.

Figure 6 is the 9-month radiograph of rabbit D-81. Only under close scrutiny can remnants of the samples be found. Again, the radiograph suggests continuing resorption of the samples.

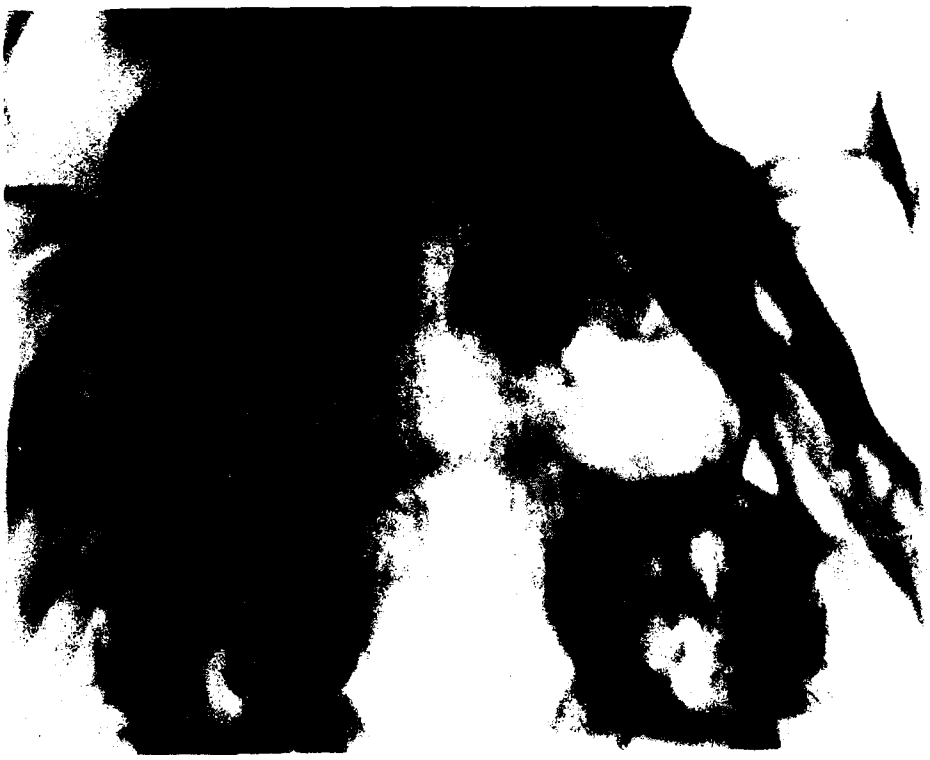

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FIGURE 3. RADIOGRAPH OF RABBIT D-81, 21 DAYS POST-IMPLANT

The implants are readily apparent in the animal's calvarium, shown as circular images. In this "live" X-ray, the directed porosity of the implants is not readily apparent.

Note that all radiographs in this research report were exposed with a step wedge included. The step wedge was used as a control to correct for any exposure differences. Consequently, all photographs of radiographs in this report are corrected, relative to a control density.

≈ 2.5X

FIGURE 4. RADIOGRAPH OF RABBIT D-81, 3 MONTHS POST-IMPLANT

This radiograph shows a decrease in overall radiodensity when compared to the previous figure. This suggests some bioresorptive activity of the implanted samples.

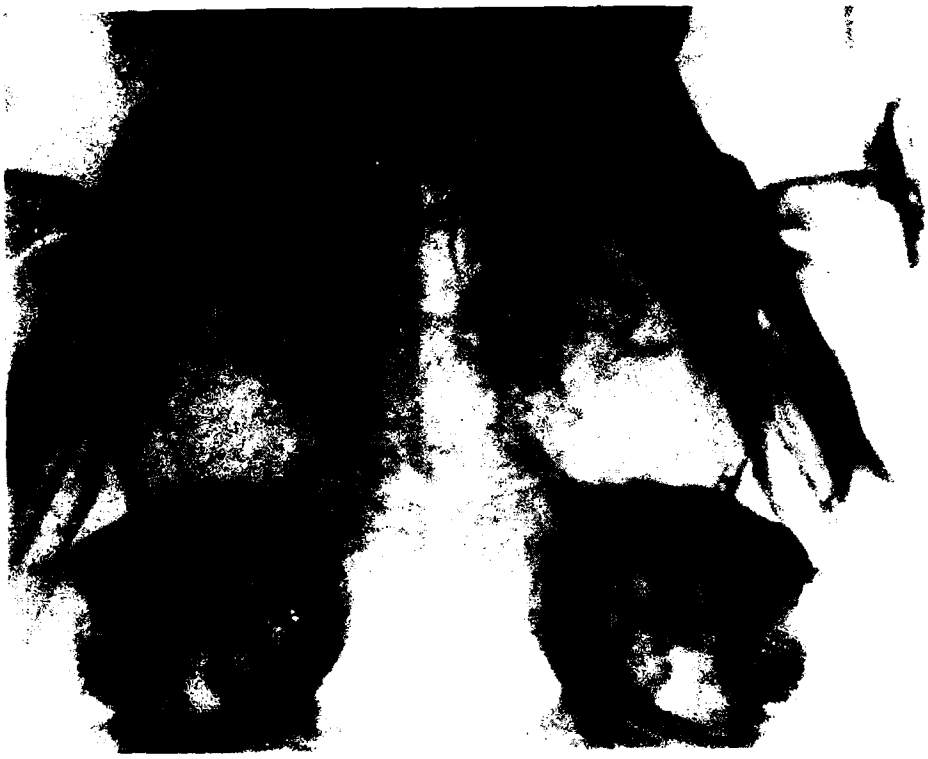

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FIGURE 5. RADIOGRAPH OF RABBIT D-81, 6 MONTHS POST-IMPLANT

This figure demonstrates a dramatic decrease in the radiodensity of these samples when compared to the previous two figures. This suggests progressive bioresorption. The density of the biomaterial now approximates that of the surrounding bone.

≈ 2.5X

FIGURE 6. RADIOGRAPH OF RABBIT D-81, 9 MONTHS POST-IMPLANT

In this figure, bioresorption is apparently extensive, remnants of the samples can be found only under close scrutiny. This is the same animal illustrated in the preceding three figures.

The same alteration in radiodensity can also be seen in another 6-month experimental animal. Figure 7 (rabbit A-81) shows the "zero time" radiograph, taken 21 days post implant. Figure 8 shows the same samples 6 months post implant. The decrease in radiodensity is not as dramatic as the previous example; however, radiodensity has significantly decreased indicating bioresorption. The directed pores coursing through the implant appear to be preferentially resorbing. Note that no loss of implant-bone integrity is evident with these unidirectional porosity specimens. It should be recalled that deleterious integrity loss was noted radiographically in previous experiments where omnidirectional rather than unidirectional porosity was utilized.

Figure 9 illustrates a three-month necropsy radiograph of an excised skull (rabbit G-81). The samples can be clearly observed in this radiograph because interfering bone and tissue structures have been removed. The same implants are illustrated below as they appeared when radiographed prior to surgery. Upon comparison, some resorption of the implants and ingrowth can be noted by widening of the directed pores and the appearance of granular material (presumably bone) within the pores. Granular-appearing material is especially noticeable in the left implant.

Figure 10 illustrates a six-month necropsy radiograph (rabbit E-81). Again, a radiograph of the same samples prior to implant is included below for comparative purposes. When compared to the pre-surgery radiograph, considerable resorption of the sample and ingrowth of bone is suggested. The degree of resorption can be readily noted in the holes normal to the film plane. Their diameter has markedly increased in the six-month experimental period.

Figure 11 represents a nine-month necropsy radiograph (rabbit D-81). Note that very significant bioresorption and bone ingrowth appears to have taken place. The change in these samples is especially dramatic when they are compared to either their control radiographs or those examples of 3- and 6-month animals. It is significant to note that there has been no loss of bone integrity. Loss of bone integrity or bone resorption when it occurs is usually noted by the radiographic appearance of large radiolucent areas.

≈ 2.5X

FIGURE 7. RADIOGRAPH OF RABBIT A-81, 21 DAYS POST-IMPLANT

This figure presents another example of directed porosity implants shortly after surgery.

≈ 2.5X

FIGURE 8. RADIOGRAPH OF RABBIT A-81, 6 MONTHS POST-IMPLANT

When compared to Figure 7, the radiodensity has decreased but not as dramatically as the previous example (rabbit G-81). In this figure, there appears to be preferential resorption along the directed pores. The pore diameters appear to be increasing.

A

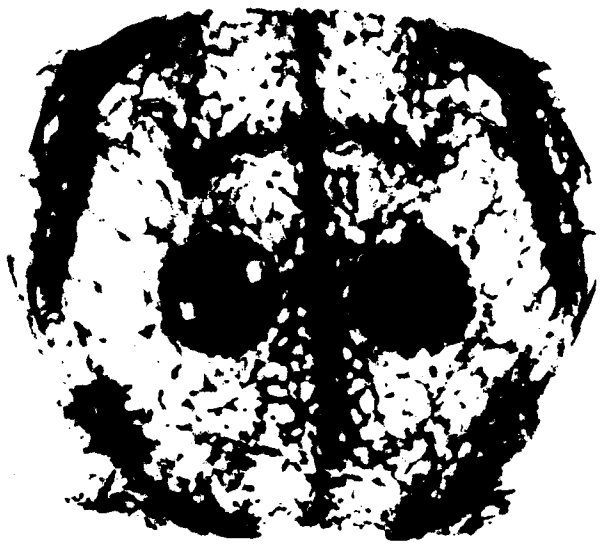
B

≈ 2.5X

FIGURE 9. THREE-MONTH POST-NECROPSY RADIOGRAPH OF EXCISED CALVARIUM FROM RABBIT G-81

In Panel A, the large directed porosity of the samples are clearly visible because the interfering bone and tissue structures have been removed. In Panel B, a radiograph of the same implants prior to surgery is presented for comparison. The identical implants are located directly above. However, the implants were rotated at surgery.

The comparison of the two radiographs shows resorption as evidenced by widening and decreased radiodensity especially along the pores, and apparent ingrowth as evidenced by granular material within the pores.



A

B

≈ 2.5X

FIGURE 10. SIX-MONTH POST-NECROPSY RADIOGRAPH OF EXCISED CALVARIUM FROM RABBIT E-81

In Panel A, the samples are shown in the excised calvarium at six months post-implant.

In Panel B, the same samples are shown prior to surgery. As in the previous example (Figure 9), bioresorption and bone ingrowth appears to be under way. When compared to Figure 9 at three months, the resorption appears to be progressing with time.

A

B

~ 2.5X

FIGURE 11. NINE-MONTH POST-NECROPSY RADIOGRAPH OF EXCISED CALVARIUM FROM RABBIT D-81

In Panel A, the samples are shown as they appear nine-months post-implant. In Panel B, the same implants are shown as they appeared prior to surgery.

In this figure, the apparent bioresorption and bone ingrowth is dramatic when compared to control, or to the 3- and 6-month samples (Figures 9 and 10). It is significant to note that there has been no bone or implant loss.

Histologic Evaluations

To evaluate the rate of ingrowth of biologic material (bone and connective tissue) into the tricalcium phosphate and the subsequent biodegradation of tricalcium phosphate, ground sections of the excised skulls were prepared. A methylmethacrylate embedding technique was used. Due to the nature of tricalcium phosphate, ground sections cannot be prepared without embedding in a rigid fixation medium such as methylmethacrylate. Sections have been prepared both pre-stained with basic fuchsin and also unstained. The histology illustrated in this report has been stained with basic fuchsin.

Figure 12 is a photomicrograph of a specimen implanted for 3 months (rabbit G-81-3L). Both transverse and longitudinal pores can be seen. Rather extensive bone formation is seen in the lower longitudinal pore. However, mostly dense connective tissue fills the upper longitudinal pore. Between the two longitudinal pores are the scalloped shapes produced by transverse pores which are extending at right angles to the plane of the figure. These pores are filled with bone, some connective tissue and diplöe-like spaces, which apparently contain marrow.

Figure 13 is a photomicrograph from another three month animal (rabbit B-81-5T). This figure presents a somewhat different appearance than the previous figure. In a longitudinal pore (shown at the bottom of the figure) bone appears to be closely adapted to the tricalcium phosphate wall of the pore, whereas the central portion of the pore appears to contain voids similar to those normally seen in the diplöe layer of calvarium bone. The diplöe is generally trabecular bone with marrow filling most of the void spaces. The old bone is at the left and bone ingrowth appears to be proceeding into the large pores. Bone within the biomaterial appears similar in morphology to the old bone.

Figure 14 is a higher magnification of the same area (rabbit B-81-5T). Note the close adaptation of bone to the tricalcium phosphate. Osteoblast-like cells appear to be lined up at the bone-biomaterial interface.

Figure 15 is a photomicrograph from a 6-month experiment (rabbit A-81-5L). All available pore spaces appear to be packed with bone or diplöe-like spaces filled with marrow. Generally, bone occupies all the available

≈ 37X

FIGURE 12. PHOTOMICROGRAPH OF TRICALCIUM PHOSPHATE SPECIMEN WITH DIRECTED POROSITY, THREE MONTHS POST-IMPLANT (RABBIT G-81-3L)

This figure shows one longitudinal pore in the lower portion of the figure with bone ingrowth. The upper longitudinal pore is filled with connective tissue. The transverse pores, seen in the middle of the figure appear as scalloped shapes. These pores are at right angles to the pore layers above and below. These pores contain bone, some connective tissue and marrow-filled spaces similar to those normally seen in the diplöe of rabbit calvaria.

Note: Included in the rabbit identification, is the slide number from which the particular photomicrograph was obtained. For example, in this figure, -3L identifies the slide number.

≈ 37X

FIGURE 13. PHOTOMICROGRAPH OF TRICALCIUM PHOSPHATE SPECIMEN WITH DIRECTED POROSITY, THREE MONTHS POST-IMPLANT (RABBIT B-81-5T)

This implant exhibits bone ingrowth preferentially at the bone biomaterial interface. The longitudinal pore at the bottom of the figure shows bone closely adapted to the tricalcium phosphate. The center of the pore contains marrow space. The bone appears to have grown from the old bone, at the left, unimpeded by the presence of the biomaterial.

≈ 90X

FIGURE 14. PHOTOMICROGRAPH OF BONE INGROWTH INTO LONGITUDINAL PORE AT HIGH MAGNIFICATION, THREE MONTHS POST-IMPLANT (RABBIT B-81-5T)

This view shows the close adaptation between bone and tricalcium phosphate. There does not appear to be a continuous connective tissue layer at this interface. Osteoblast-like cells appear lined up at the bone-biomaterial interface.

≈ 37X

FIGURE 15. PHOTOMICROGRAPH OF BONE INGROWTH INTO TRICALCIUM PHOSPHATE, SIX MONTHS POST-IMPLANT (RABBIT A-81-5L)

At six months, all available spaces within the implant appear densely packed with bone and marrow spaces. As seen previously bone is predominantly seen at the biomaterial interface.

biomaterial interface space. Figure 16 is a photomicrograph of the contralateral tricalcium phosphate implant (rabbit A-81-5T). Again, bone and diplöe marrow spaces are seen filling the available biomaterial pore space. However, in this sample it appears that considerably more bioresorption has taken place as evidenced by the thin spicules of tricalcium phosphate that remain.

Figure 17 is a high magnification photomicrograph from a six-month implant specimen (rabbit B-81-4T). In this case, a transverse pore is shown. As noted previously, bone appears to adapt closely to the biomaterial whereas the central portion of the pore is filled with a non-osseous, diplöe-like space filled with marrow.

The histologic specimens prepared for this project indicate progressive bone ingrowth, and apparently simultaneous resorption of directed porosity tricalcium phosphate without bone loss or loss of implant mechanical integrity.

CONCLUSIONS AND DISCUSSION

This study further indicates that the use of longitudinally organized unidirectional pores is a viable method to prevent the deleterious loss of implant and bone integrity. Such loss of integrity was seen in previous experiments when omnidirectional materials were studied. This loss of integrity has continually plagued the use of omnidirectional structural bioresorbable bone scaffold materials. The omnidirectional design has two major drawbacks. First, the strength of the material is severely limited. Second, and more importantly, as bone grows in and the material simultaneously biodegrades, mechanical integrity of the biomaterial-bone complex is usually lost. The result is a bone loss and filling of the area with connective tissue in response to loss of stability. With the unidirectional material presented in this report, the use of relatively large channels for bone to grow through, coupled with a stronger wall material, appears to provide adequate mechanical integrity during the resorption process to prevent bone loss.

At the time of this report, animal experiments have been completed up through month nine. Radiologically, extensive resorption of tricalcium phosphate has taken place without loss of bone integrity. Histologically

≈ 37X

FIGURE 16. PHOTOMICROGRAPH OF BONE INGROWTH INTO TRICALCIUM PHOSPHATE, SIX MONTHS POST-IMPLANT (RABBIT A-81-5T)

This figure demonstrates extensive bioresorption of the tricalcium phosphate. The walls of biomaterial between the pores have been reduced to spicules. As in previous figures all available space is packed with bone and/or marrow space. The consistency of the bone approximates that of normal calvarium.

≈ 90X

FIGURE 17. PHOTOMICROGRAPH OF TRICALCIUM PHOSPHATE SIX MONTHS POST-IMPLANT (RABBIT E-81-4T)

This figure at high magnification shows the close adaptation of bone to the biomaterial. The periphery of the pore contains dense bone, whereas the central pore area contains marrow space.

specimens up to six months were available for observation. Extensive bone ingrowth was seen especially at six months. In some areas the resorption is advanced to the stage that interconnecting bridges between the various channels have resorbed. On the basis of data available to date, the bulk of bone formed appears adequate to structurally support the area, since no loss of integrity is seen. This observation is based only upon 9 month radiographic data. It should be recalled that loss of structural integrity was observed in 9- and 12-month samples with omnidirectional porosity material. Consequently, this observation cannot be confirmed until 9 month histologic data is available. At this time, no negative observations have been made.

The outcome for successful healing of the bone void via scaffold ingrowth and resorption appears favorable. The biomaterial being used appears to be adequate in overall design. The basic methodology of material construction appears to be practical for volume production. This method will allow for the construction of numerous variations based upon the same basic design concept. One can envision materials designed for various bone replacement uses within the body. Pore direction can be manufactured as desired by the user. Significant increases in structural properties of this material relative to previous material can be anticipated.

The present biomaterial is less than optimal in two areas: first, the bonding between the laminated sheets of material is less than desirable. The present material can be separated at the lamination boundaries by probing. Secondly, the density of the bulk material is less than desired. This low-bulk density reduces the overall strength of the biomaterial. Too much microporosity in the bulk material will additionally increase the bioresorption rate, which may negatively effect the end result.

RECOMMENDATIONS

The concept of directed porosity has now been shown viable in three different experimental series. The last of the series will shortly be completed utilizing material possessing close to the desired physical configuration. Research efforts should now be directed towards improving the mechanical properties of the material. The bulk density of the material and the

interlayer bonding should be improved by evaluating improved processing technologies. Once an improved material is available, *in vivo* evaluations should be performed at Battelle-Columbus and dog mandible experiments at USAIDR.

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